

1/11/93

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Attention: Vittorio Sgaramella
 Department of Microbiology and Genetics
 University of Pavia

From: Greg Tomblin
 Rockefeller University, Lederberg lab

Dear Vittorio,

Since my last letter I have been trying to clarify the fingerprinting on the mutants and have the sequence side by side for both P3 and P4 the last gel was beautifully resolved but unfortunately ripped and folded right at the spot of the mutations. The gel consisted of the new protocol which I mentioned to you before, ie. using 8% Hydrolink with 40% formamide and normal (not wedged) spacers. I believe that the samples were overheated the last time I used this method - the lanes appeared blurry. So, in order to avoid this I heat the samples for approx. 5-7 minutes, then chill on ice approx. same time, then load. This last gel was really nice, but just hard to handle. We still have more of the samples.. good sequencing with sequenase, and fingerprinting done with 32P instead of 35S - was extracted and precipitated. So, I will do my best to make the perfect gel and handle it most carefully. From what I could see there were no gross/ indirect effects of the mutations on the fingerprint for at least P3, but P4 might be slightly more complicated. Don't lose hope, I really think that we are almost there. I'm making another gel today with wedged spacers but 8% with 40% formamide and will run it tomorrow (Tues 1/12/93), so we should have something Wed. ... this gel runs approx. 7 to 8 hours..... patience...

In the arena of endonuclease..... I haven't been able to do very much since our glass plates have been limited and I've wanted to knock off the fingerprinting.... Have you found anything new?

In David's arena things have been rough. I am trying to finish controls . will let you know. We also have a new member- Sri Sastri has joined , so we're trying to make space.

So for now, Happy New Year. Non Prevalabunt....

I'm back.... it's monday 1/25/93 now. The last three gels have failed in the sense that they were all blurry, although counts were incorporated. I have been experiencing real difficulty with handling the gels in general.. they have been sticking to the plates. I suspect that the formamide gels might be the root of the problem.....but it worked really well at least once. It has been very disappointing. On friday we received some new glass plates, that is to say, we are no longer limited by plates, so I will run two gels, one 6% acrylamide without formamide, and another with Hydrolink + formamide. The reactions should still be good - they are only a few days old. Meanwhile I will also take the autoradiograph of the nice gel (Hydrolink + formamide; which ripped in the immediate area of the mutations) to the media services and send you a copy- to show you how nice this type of gel resolves (with no compressions of "G" bands and very little smiling) so you can appreciate why I am trying to use this technique. I am really sorry that it has been taking so long, as I said it is really frustrating to put in so many hours for nothing.

I am hoping that the controls for David's experiments will yield some conclusive data. I have finally found a way to destroy the RNA which was supposedly DNAsed after transcription (alkali then dialyzing on a .025micrometer millipore membrane) and have also shown that DNA primers do not slip through-- then the synthesis is not hindered- this is to show that the DNase treatment after the transcription is complete and that the supposed RNA generated mutants are not the result of any DNA (of the PCR product which the RNA was transcribed off) which might have survived. We ran out of ssccc uracil containing phagescript (Template for the synthesis reactions) - and generating new, clean, homogeneous stuff has been time consuming as well- but I think that we have some now. As for the RNA primer in a PCR, it's been on hold, but plan to start soon again as well as making 30 and 60 base RNA to use in the mutagenesis expts as well as RNA PCR.

Julie has started some good experiments involving ELISA in the detection of RNA/DNA hybrids, as well as RecA protein with antibodies, so far with nice looking controls.

Helene is back from France and starting expts which I believe correlate in vivo and in vitro studies of formaldehyde mutagenesis using phagescript as the substrate. I am sure that Dr. Lederberg will keep you up to date with it.

David has been following up the analysis of his RecD mutants which are highly resistant to ds breaks and I' sure has a ton of data.... it should be very interesting.

Ken met with some success in purifying his heteroduplexes which he made by PCR, and started transcribing off of them.... this should also be interesting. Right now he's trying to resolve things with Quin ling - he has started the divorce proceedings, and has attempted to bring her back to China. We wish him luck, he has seemed somewhat less stressed.

Mike has had some interesting ideas which he will probably tell you about- involving organization of databases in relation to the Human Genome Project and organizing molecular genetic info in general. He is also working on gel analysis with Mic, and a possible spreadsheet format for looking at a PCR reaction....

As I said, Srinivas Sastri has arrived and is settling in-- no bench work yet...

We have had a couple of good seminars... Bruce Ames spoke and also visited the lab. I also attended a seminar entitled " From the crime scene to the courtroom, and the biology of forensic medicine" -- mentioned were RFLP analysis which although out of date? is the most commonly used technique by the FBI.... fingerprinting by other means was only briefly mentioned.. Anyway, today Sir Walter Bodmer will give the Joshua Lederberg lecture in molecular genetics on human polyposis and mutation rates in humans- should be a good one.

As for my love life, things are great. Robin sends her best. So for the moment, stay well , will let you know what happens.

A handwritten signature in cursive script, appearing to read "Greg", with a horizontal line underneath.